[0026] The IPC nucleic acid molecule of the present invention may be used as a universal internal control as it comprises unique primer and probe sites and does not exhibit homology with any known nucleic acid sequences that may interfere with this assay, i.e. does not anneal with known nucleic acid sequences during conventional PCR techniques.

[0027] The IPC nucleic acid molecule of the present invention provides greater flexibility over commercially available IPCs as a variety of reporter molecule and quencher molecule pairs may be used and since the primers and IPC nucleic acid molecule sequences are independent, various concentrations of each may be used.

[0028] As described herein, the 153 base pair (bp) product from a *Bacillus anthracis* Protective Antigen (PA) PCR assay developed by the Diagnostic Systems Division (DSD) at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) was used (publication in progress). The 153 bp sequence is:

(SEQ ID NO:1)
5' TTCAAGTTGT ACTGGACCGA TTCTCAAAAT AAAAAAGAAG

TGATTTCTAG TGATAACTTA CAATTGCCAG AATTAAAACA

AAAATCTTCG AACTCAAGAA AAAAGCGAAG TACAAGTGCT

GGACCTACGG TTCCAGACCG TGACAATGAT GGA 3'.

[0029] The probe and both primer sites were mutated to pre-determined sequences as follows:

Upper Primer: IPC3L
(SEQ ID NO:2)
5' CGT TGT TAC CGA CTG GAT TAT TAC C 3';

Lower Primer: IPC5U
(SEQ ID NO:3)
5' TCC GCA TAC CAG TTG TTG TCG 3' and

Probe: IPCP35F
(SEQ ID NO:4)
5' CGT AGT TGA TCG CTC TCA GTC CAT CCG T 3'.

[0030] The original sequences were randomized and the random sequences were checked with a nucleotide BLAST search to confirm their uniqueness. The original sequences of the PA assay were as follows:

Upper Primer: BAPA3U
(SEQ ID NO:5)
5' TTC AAG TTG TAC TGG ACC GAT TCT C 3';

Lower Primer: BAPA5L
(SEQ ID NO:6)
5' tcc atc att gtc acg gtc tgg 3'; and

Probe: BAPA3P2A
(SEQ ID NO:7)
5' CCG TAG GTC CAG CAC TTG TAC TTC GCT T 3'.

[0031] The probe site was mutated first, followed by the upper primer site and then finally the lower primer site. As disclosed in Example 1, the mutations were conducted with PCR-based site directed mutagenesis methods known in the art. See Courtney, B. C., et al. (1999) Analytical Biochemistry 270:249-256. The methods were the same for all three sites, the only differences were the mutagenic oligonucleotide sequences.

[0032] Generally, mutations of each site were performed in three stages comprising five steps. For the initial probe mutation, genomic *Bacillus anthracis* DNA was used, and for the subsequent primer mutations, the plasmid DNA from the clone of the previous mutation was used. Mutagenic oligonucleotides were used to introduce the desired mutations. For round 1, these oligos contained ½ the sequence of *B. anthracis* genomic DNA and ½ the sequence of the desired mutation. These mutagenic oligos were paired up with an oligo outside of the final 153 bp PA product. When amplified with PCR, the result was two products each containing half of the final desired mutation sequence. For round 2, these mutagenic oligos consisted of ½ the new sequence that was introduced in round 1 and ½ the sequence of the rest of the desired mutation. The two products from round 1 were used as templates. Again, these mutagenic oligos were paired up with an oligo outside of the final 153 bp PA product. When amplified with PCR, the result was two products each containing all of the final desired mutation sequence. Finally in round 3, the two products from round 2 were used as primers on each other and ligated together, in addition the two oligos outside of the 153 bp product were used to further amplify it and increase the copy number of the final product. This final product was ligated into the pCR2.1 vector (Invitrogen Corporation, Carlsbad, Calif.) and transformed into competent INVαF'*E. coli* (Invitrogen Corporation, Carlsbad, Calif.).

[0033] For round 1 of the probe mutation, the template DNA used was 1 ng of Ames genomic DNA (USAMRIID, Ft. Detrick, Md.), and the primers were: BANPAIS1

BANPAIS1
(SEQ ID NO:8)
5' GTA ACA ATG TGG GTA GAT GAC C 3'

PA35PC1L
(SEQ ID NO:9)
5' TCT CAG TCC ATC CGT TTT TCT TGA GTT C 3'

[0034] The product, Fragment 1, was a 252 bp product as follows:

(SEQ ID NO:10)
5''GTAACAATGTGGGTAGATGACCAAGAAGTGATTAATAAAGCTTCTAA

TTCTAACAAAATCAGATTAGAAAAAGGAAGATTATATCAAATAAAAATTC

AATATCAACGAGAAAATCCTACTGAAAAAGGATTGGATTTCAAGTTGTAC

TGGACCGATTCTCAAAATAAAAAAGAAGTGATTTCTAGTGATAACTTACA

ATTGCCAGAATTAAAACAAAAATCTTCGAACTCAAGAAAAACGGATGGAC

TGAGA 3'.

[0035] Also for round 1 of the probe mutation, the template DNA used was 1 ng of Ames genomic DNA (USAMRIID, Ft. Detrick, Md.), and the primers were:

BANPAIA1
(SEQ ID NO:11)
5' CTT ATC AAT CCG TCC TGT AAC C 3'

PA35PC1U
(SEQ ID NO:12)
5' GCG ATC AAC TAC GTT CCA GAC CGT G 3'